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(19)

(54) METHOD OF PREPARING 2H-TETRAZOLIUM CHLORIDES AND 2H-TETRAZOLIUM CHLORIDES HYDROCHLORIDES

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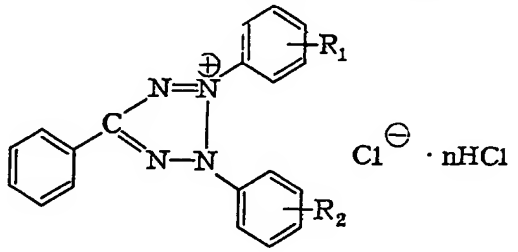
This invention relates to a method of preparing 2H-tetrazolium chlorides and 2H-tetrazolium chlorides hydrochlorides.

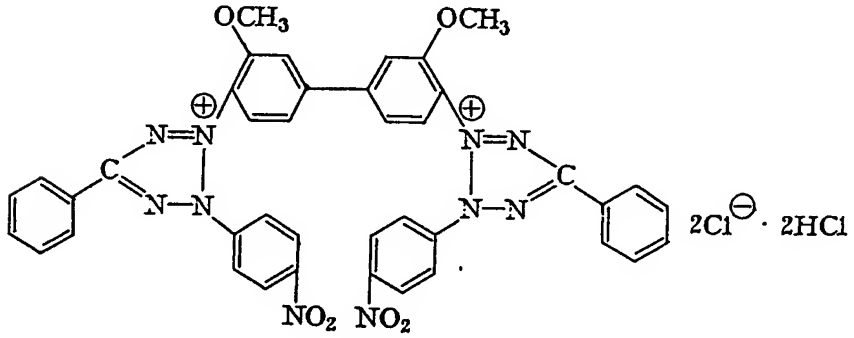
Such 2H-tetrazolium salts are used in biochemistry and agrochemistry for studying enzymes of the oxidation-reduction metabolism, of the dehydrogenase type, in plant tissues, eye lenses, cell cultures, blood cells, blood serum, tumours, bacteria, salmonellae causing abdominal fever and mouse typhoid; animal organs, and in soil and effluents. Moreover, such salts are used in germination and viability tests for the seeds of for example cotton, corn, and wheat, for the determination of boron hydrides in air, and also for histochemical studies of corn.

The present invention provides a method of preparing 2H-tetrazolium chlorides and 2H-tetrazolium chlorides hydrochlorides having the general formula



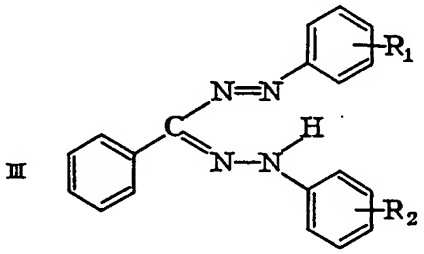
I

where R_1 and R_2 are each H, OH, NO_2, Cl, or OCH_2COOH, and n is from 0 to 3, or the 2H-tetrazolium chloride hydrochloride having the formula



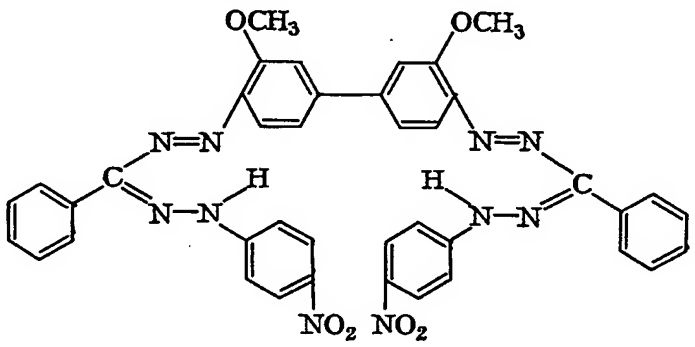
II

comprising oxidizing a formazan having the general formula



III

where R_1 and R_2 are as specified above, or a formazan having the formula



IV

with chlorine in a medium of a polar organic solvent at a temperature from −5 to +20°C, chlorine being introduced into the said solvent in the gaseous state, the molar ratio of formazan to chlorine being from 1:1 to 1:10 and isolating the end product when the oxidation process is completed.

The method according to the invention of oxidizing formazans precludes formation of resins or decomposition of a considerable portion of the product; for example, the yield of 2,3,5-triphenyltetrazolium chloride is as high as 80 per cent. The time of the synthesis is reduced from 10 hours to 10—60 minutes by the method according to the invention.

In order to obtain the end product in crystal form, a solvent miscible with water is preferably used as the polar organic solvent, and the oxidation process is carried out in the presence of sodium hydroxide taken in a quantity equimolar with respect to formazan.

The starting formazan may be suitably 1,3,5 - triphenylformazan, 1 - (4 - nitrophenyl) - 3,5 - diphenylformazan, 1 - (2 - carboxymethoxy - 4 - nitrophenyl) - 3-phenyl - 5 - (2 - carboxymethoxyphenyl) - formazan, 1,2 - (diphenyl) - 5 - (4-chloro)phenyl - formazan, or the dinitrodimethoxydiformazan of formula (IV).

The polar organic solvent may be suitably methyl alcohol, ethyl alcohol, propyl alcohol, toluene, methyl acetate, or ethyl acetate. Of these, ethyl alcohol, methyl alcohol and ethyl acetate are the most preferred.

To increase the yield of the end product to 90 per cent (the assay being not less than 96 per cent), absolute ethyl alcohol is preferably used as the polar organic solvent.

In order to purify the obtained 2H-tetrazolium chloride hydrochloride from iron salts, and also to decrease the hydrogen chloride content of the salt, the obtained 2H-tetrazolium chloride hydrochloride may be suitably treated, at the stage of isolation, with aqueous ammonia to pH 8.

The method according to the invention makes it possible to convert 2H-tetrazolium chloride hydrochloride, obtained in the process of oxidation, into 2H-tetrazolium chloride. To this end 2H-tetrazolium chloride hydrochloride is preferably treated, at the stage of isolation, with a solution of potassium hydroxide in ethyl alcohol, the ratio of the alkali to the hydrochloride being equimolar.

The duration of the synthesis is reduced two or three times if the oxidation process is effected in a reactor, provided with a diffuser and a turbine agitator, made out of titanium, instead of glass as is the case with laboratory equipment.

The method according to the invention may be suitably carried out as follows.

Equimolar quantities of sodium hydroxide and formazan are mixed in absolute ethyl alcohol (the weight ratio of formazan to ethyl alcohol being from 1:15 to 1:25), gaseous chloride being blown through the ethyl alcohol (the molar ratio of formazan to chlorine being from 1:2 to 1:5) at a temperature from −5 to +20°C. When the reaction mixture becomes colourless, the reaction is completed. The end product is then isolated by any suitable method, e.g. by mixing the reaction mixture with activated carbon, filtering, evaporating the filtrate, extracting with water, and evaporating the aqueous extract to crystallize the end product, 2H-tetrazolium chloride hydrochloride.

The invention will be further described with reference to the following illustrative Examples.

Example 1

Preparation of 2,3,5-triphenyltetrazolium chloride hydrochloride.

5 g (0.015 M) of 1,3,5-triphenylformazan were dissolved in 100 ml of ethyl acetate in a round-bottomed flask while blowing in 1 litre (0.043 M) of chlorine gas at a temperature of 0°C for an hour. The reaction mixture was transferred into 1 litre of absolute diethyl ether. The amorphous product was re-precipitated several times from ethyl alcohol with diethyl ether, and dried in a vacuum desiccator. The yield of the end product was 4.2 g (70 per cent). The assay was 96 per cent.

Found, in per cent: C 60.20, 59.87; H 4.70, 4.45; Cl 16.46, 16.70; N 14.65, 14.53. $C_{19}H_{15}N_4Cl.0.75HCl \cdot H_2O$.

Calculated, in per cent: C 60.00, H 4.71, Cl 16.30, N 14.75.

Example 2

Preparation of 2,3,5-triphenyltetrazolium chloride hydrochloride.

5 g (0.015 M) of 1,3,5-triphenylformazan were dissolved in 100 ml of absolute ethyl alcohol in a round-bottomed glass flask while intensely stirring the mixture. 0.96 litres (0.043 M) of chlorine gas were passed in at a temperature of 20°C for 40 minutes until the red colour disappeared. The obtained solution was mixed with 0.5 g of activated carbon, and then filtered, and the filtrate added to 1 litre of diethyl ether to precipitate 4.5 g of amorphous 2,3,5-triphenyltetrazolium chloride hydrochloride. The yield of the end product was 75 per cent.

The second half-wave potential, characterizing the reduction of the end product to formazan, $E_{1/2}$ was −0.7 V.

Found, in per cent: C 60.20, 59.87; H 4.70, 4.45; Cl 16.46, 16.70; N 14.65, 14.53. $C_{19}H_{15}N_4Cl \cdot 0.75HCl \cdot H_2O$.

Calculated, in per cent: C 60.00, H 4.71, Cl 16.30, N 14.75.

At a temperature of 150—165°C the hydrochloride was split off, the process being accompanied by foaming of the powdered salt in a capillary. At a temperature of 227—228°C the powder melted with decomposition.

Example 3

Preparation of 2,3,5-triphenyltetrazolium chloride.

The 2,3,5-triphenyltetrazolium chloride hydrochloride obtained in Example 2 was dissolved in ethyl alcohol and treated with a solution of potassium hydroxide in ethyl alcohol. The ratio of the alkali to the hydrochloride was equimolar. The solution was filtered, and the filtrate poured into diethyl ether. The product was separated on a filter. The obtained end product melted at 230—231°C with decomposition.

The second half-wave potential, at which the end product was reduced to formazan, $E_{1/2}$ was −0.73 V.

Found, in per cent: C 64.34, 64.15; H 4.44, 4.33; Cl 10.00, 10.04; N 15.92. 15.80. $C_{19}H_{15}N_4Cl \cdot H_2O$.

Calculated, in per cent: C 64.40, H 4.85, Cl 10.04, N 15.88.

Example 4

Preparation of 2,3,5-triphenyltetrazolium chloride trihydrochloride.

The procedure was the same as described in Example 2, except that the quantity of chlorine gas used was 3.5 litres (0.15 M). The yield of the end product was 5.0 g (69 percent).

Found, in per cent: Cl 32.15, 31.98. $C_{19}H_{15}N_4Cl \cdot 3HCl$

Calculated, in per cent: Cl 32.35

As the product was recrystallized from dimethyl formamide, the hydrochloride was split off.

Example 5

Preparation of 2,3,5-triphenyltetrazolium chloride hydrochloride.

5 g (0.015 M) of 1,3,5-triphenylformazan and 0.7 g (0.015 M) of sodium hydroxide were mixed in 100 ml of absolute ethyl alcohol and 0.96 litres (0.042 M) of chlorine gas were passed in for 45 minutes at a temperature of −5°C. Activated carbon was added, the components mixed, the mixture passed through a filter, the filtrate evaporated, and the residue dissolved in water, followed by treatment with activated carbon, filtering, and evaporation of the filtrate. The obtained crystals were white with a creamy tint. The yield of the end product was 4.8 g (85 per cent).

Example 6

Preparation of 2,3,5-triphenyltetrazolium chloride hydrochloride.

The procedure was the same as described in Example 5, except that the temperature of the reaction was −1°C, the time during which chlorine gas was passed was 35 minutes, and methyl alcohol was used as the polar organic solvent. The yield of the end product was 4.8 g (85 per cent). The assay was 92 per cent.

Example 7

Preparation of 2,3,5-triphenyltetrazolium chloride hemihydrochloride.

25 g (0.08 M) of 1,3,5-triphenylformazan, 550 ml of absolute ethyl alcohol, and 3.64 g (0.086 M) of sodium hydroxide were mixed in a glass flask provided with a glass stirrer and 4.8 litres (0.21 M) of chlorine gas were passed in for 35 minutes at a temperature of −1°C. Activated carbon was added to the reaction mixture containing 2,3,5-triphenyltetrazolium chloride hydrochloride (n=0.75), the mixture was stirred and filtered, the filtrate evaporated, the residue extracted with water, and the aqueous extract was treated with activated carbon and filtered. The filtrate was treated with aqueous ammonia to pH 8, and then treated with activated carbon and filtered, and the filtrate evaporated. The yield of the end product was 86 per cent. The assay of 2,3,5-triphenyltetrazolium chloride hemihydrochloride (n=0.5) was 9 per cent. The crystalline product had a creamy tint.

Example 8

Preparation of 2,3,5-triphenyltetrazolium chloride hemihydrochloride.

The procedure was the same as described in Example 7, except that a reactor with a diffuser and a turbine agitator, made of titanium was used instead of a glass reaction flask and a glass stirrer. The oxidation continued for 18 minutes. The yield of the crystalline end product was 83 per cent. The assay was 96 per cent.

Example 9

Preparation of 2 - (2 - carboxymethoxyphenyl) - 3 - (2 - carboxymethoxy - 4-nitrophenyl) - 5 - phenyltetrazolium chloride hemihydrochloride.

5 g (0.01 M) of 1 - (2 - carboxymethoxy - 4 - nitrophenyl) - 3 - phenyl - 5 - (2 - carboxymethoxyphenyl) - formazan and 120 ml of absolute ethyl alcohol were mixed in a glass beaker and 1.2 litres (0.05 M) of chlorine gas were passed in at a temperature of 0°C for 20 minutes. 0.5 g of activated carbon were added to the reaction mixture, followed by mixing for 15 minutes, and filtering. The filtrate was transferred into 4 litres of diethyl ether, and the precipitate separated, followed by reprecipitation from ethyl alcohol with diethyl ether. The yield of the end product was 4.2 g (75 per cent).

Found, in per cent: C 49.14, 48.70; H 3.38, 3.37; Cl 9.98, 9.72; N 11.78, 12.0. $C_{23}H_{18}N_5O_8Cl \cdot 0.5HCl \cdot H_2O$.

Calculated, in per cent: C 48.98, H 3.66, Cl 9.43, N 12.41.

Example 10

Preparation of 2,5-diphenyl-3-(4-nitrophenyl)-tetrazolium chloride hydrochloride.

5 g (0.0145 M) of 1 - (4 - nitrophenyl) - 3,5 - diphenylformazan, 0.58 g (0.0145 M) of sodium hydroxide, and 100 ml of absolute ethyl alcohol were mixed in a glass beaker and 1 litre (0.043 M) of chlorine gas was passed in at a temperature of 0°C for an hour. The reaction mixture containing 2,5-diphenyl-3-(4-nitrophenyl)-tetrazolium chloride hydrochloride (n=0.75) was treated with activated carbon and filtered, the filtrate evaporated, the residue extracted with 100 ml of distilled water at a temperature of 70°C, and the aqueous extract treated with activated carbon and filtered again. The filtrate was treated with aqueous ammonia to pH 8, and the solution treated with activated carbon and filtered, and the filtrate evaporated. The crystalline precipitate was dried, and the crystals ground and re-precipitated from absolute ethyl alcohol with

diethyl ether. The yield of the crystalline end product was 4.4 g (80 per cent). The assay was 98 per cent. The product melted at 210—212°C with decomposition.

Found, in per cent: C 56.44, 55.82; H 3.88, 3.82; N 17.6, 17.64; Cl 11.4, 11.54. $C_{19}H_{14}N_5O_2Cl \cdot 0.25HCl \cdot 0.75H_2O$

Calculated, in per cent: C 56.5, H 3.92, N 17.4, Cl 11.03.

Example 11

Preparation of 2 - (2 - hydroxyphenyl) - 3 - (2 - carboxymethoxyphenyl) - 5-phenyltetrazolium chloride hydrochloride.

5 g (0.012 M) of 1 - (2 - carboxymethoxyphenyl) - 5 - phenyl - 5 - (2 - hydroxyphenyl) - formazan were mixed with 100 ml of absolute ethyl alcohol and 0.9 litres (0.04 M) of chlorine gas were passed in at a temperature of −5°C for ten minutes until the red colour disappeared. 0.5 g of activated carbon were added to the reaction mixture which was stirred for 15 minutes. The carbon was separated by filtration, and 2-(2-hydroxyphenyl)-3-(2-carboxymethoxyphenyl)-5-phenyltetrazolium chloride hydrochloride precipitated with diethyl ether. The yield was 5.4 g (96 per cent). The product was reprecipitated from absolute ethyl alcohol with diethyl ether (1:6). The yield was 4 g (71 per cent).

Found, in percent: C 54.14, 54.65; H 3.98, 3.98; N 11.15, 11.1; Cl 10.2, 10.87. $C_{21}H_{16}N_4O_4 \cdot 1.5HCl \cdot 0.5C_2H_5OH \cdot H_2O$

Calculated, in per cent: C 54.59, H 4.67. N 11.56, Cl 10.98.

The second half-wave potential, at which the product was reduced to formazan, $E_{1/2}$ was −1.25 V.

Example 12

Preparation of 2-(4-chloro)phenyl-3,5-diphenyltetrazolium chloride hydrochloride.

3 g (0.01 M) of 1,3-(diphenyl)-5-(4-chloro)-phenylformazan were mixed with 60 ml of absolute ethyl alcohol and 0.6 litres (0.03 M) of chlorine gas were passed in at a temperature of from −2 to −0°C for ten minutes. 0.3 g of activated carbon were added followed by mixing for 15 minutes. The carbon was separated by filtration, and the product was precipitated from the filtrate with diethyl ether, followed by reprecipitation twice from absolute ethyl alcohol with diethyl ether. The yield of the white powdered product was 2g, which corresponded to 50%. The assay of 2-(4-chloro)-phenyl-3,5-diphenyltetrazolium chloride hydrocarbon was 98 per cent.

Found, in per cent: C 54.92, H 3.80, N 13.29, Cl 23.70.
$C_{19}H_{14}N_4Cl_2 \cdot 0.75HCl \cdot H_2O$.

Calculated, in per cent: C 55.05, H 4.06, N 13.51, Cl 23.52.

Example 13

Preparation of 2,2′ - di - (4 - nitrophenyl) - 5,5′ - diphenyl - 3,3′ - (3,3′ - dimethoxy - 4 - 4′ - diphenylene) - ditetrazolium chloride hydrochloride (nitrotetrazolium blue).

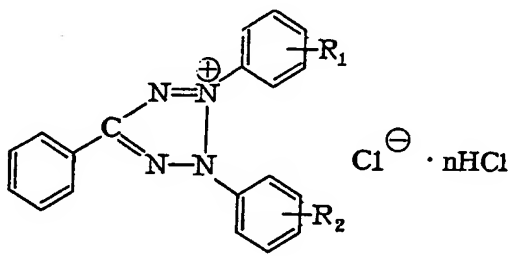
The procedure was the same as described in Example 5, except that dinitrodimethoxy diformazan in an amount of 3.7 g (0.005 M) was used as the initial formazan. The end product was reprecipitated from ethanol by diethyl ether (1:10). The yield of the end product was 3.7 g (90%) and was in the form of slightly yellow crystals.

Found, in per cent: N 14.25, Cl 14.70. $C_{40}H_{30}N_{10}O_6Cl_2 \cdot 2HCl \cdot 4H_2O$.

Calculated, in per cent: N 14.55, Cl 14.74.

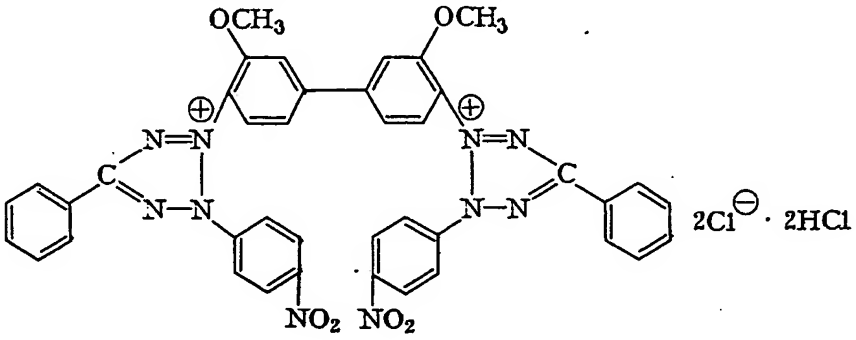
WHAT WE CLAIM IS:—

1. A method of preparing 2H-tetrazolium chlorides and 2H-tetrazolium chlorides hydrochlorides having the general formula



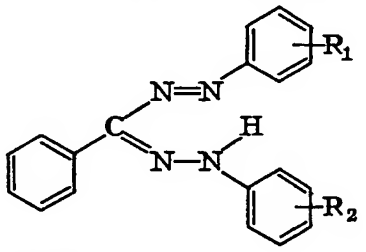
I

where R_1 and R_2 are each H, OH, NO_2, Cl, or OCH_2COOH, and n is from 0 to 3, or the 2H-tetrazolium chloride hydrochloride having the formula



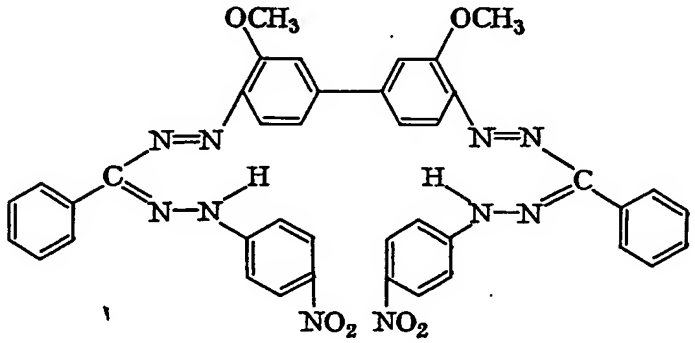
II

comprising oxidizing a formazan having the general formula



III

where R_1 and R_2 are as specified above, or a formazan having the formula



IV

with chlorine in a medium of a polar organic solvent at a temperature from −5 to +20°C, chlorine being introduced into the said solvent in the gaseous state, the molar ratio of formazan to chlorine being from 1:1 to 1:10, and isolating the end product when the oxidation process is completed.

2. A method as claimed in claim 1, in which the polar organic solvent is a solvent miscible with water, and the oxidation process is carried out in the presence of sodium hydroxide taken in a quantity equimolar with respect to formazan.

3. A method as claimed in claim 1 or 2, in which the formazan is 1,3,5 - triphenylformazan, 1 - (4 - nitrophenyl) - 3,5 - diphenylformazan, 1 - (2 - carboxymethoxy - 4 - nitrophenyl) - 3 - phenyl - 5 - (2 - carboxymethoxyphenyl) - formazan, 1 - (2 - carboxymethoxyphenyl) - 3 - phenyl - 5 - (2 - hydroxyphenyl) - formazan, 1,3 - (diphenyl) - 5 - (4 - chloro) - phenyl - formazan, or the dinitrodimethoxydiformazan of formula (IV).

4. A method as claimed in any of claims 1 to 3 in which the polar organic solvent is ethyl alcohol, methyl alcohol, or ethyl acetate.

5. A method as claimed in any of claims 1 to 4 in which the obtained 2H-tetrazolium chloride hydrochloride is treated, at the stage of isolation, with aqueous ammonia to pH 8.

6. A method as claimed in any of claims 1 to 5, in which the obtained 2H-tetra-

zolium chloride hydrochloride, at the stage of isolation, is converted into 2H-tetrazolium chloride, by treating it with a solution of potassium hydroxide in ethyl alcohol,

7. A method as claimed in any of claims 1 to 6, in which the oxidation process the ratio of the alkali to the hydrochloride being equimolar.

is carried out in a reactor, provided with a diffuser and a turbine agitator made of titanium.

8. A method of preparing 2H-tetrazolium chlorides and 2H-tetrazolium chlorides hydrochlorides substantially as herein described in any of the foregoing Examples.

9. 2H-tetrazolium chlorides and 2H-tetrazolium chlorides hydrochlorides when prepared by the method claimed in any of claims 1 to 8.

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